Dry Heat and Hot Water Treatments for Disinfesting Cottonseed of *Fusarium oxysporum* f. sp. *vasinfectum*

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ABSTRACT

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The potential of low- and high-temperature dry heat, and hot water treatments, for disinfesting cottonseed of *Fusarium oxysporum* f. sp. *vasinfectum* was investigated. Naturally infected seeds from Louisiana were air-heated at 30, 35, and 40°C for up to 24 weeks. Seed harvested from bolls inoculated with race 4 of *F. oxysporum* f. sp. *vasinfectum* were incubated in dry heat at 60, 70, and 80°C for 2 to 14 days, or were immersed in 90°C water from 45 s to 3 min. The effects on seed germination and vigor of hot water treatment and a subset of the high-temperature dry heat treatments were also examined in seeds of a Pima (*Gossypium barbadense*) and an Upland (*G. hirsutum*) cultivar. Low- or high-temperature dry heat did not eliminate *Fusarium* spp. from the seed, although seed infection declined more rapidly with higher incubation temperatures. High-temperature dry heat treatments effective in eliminating fusaria also significantly reduced seed vigor in both the Pima and Upland cultivars. Seed from all times of immersion in hot water were less frequently infected with *Fusarium* spp. than nontreated seed. Incidence of seed infection did not differ significantly among immersion times ranging from 75 s to 3 min. Immersion in 90°C water did not reduce germination or vigor at exposure times ≤120 s and ≤150 s for seeds of Pima and Upland cotton, respectively. Results from the hot water treatments suggest that thermotherapy may be optimized to provide a tactic to prevent the spread of virulent *F. oxysporum* f. sp. *vasinfectum* genotypes into uninfested areas through infected seed.

Recent developments in the Fusarium wilt pathosystem of cotton (*Gossypium* spp.) cause concern about the potential risks posed by the unintended dissemination of infected seed. In the last 15 years, novel genotypes of the pathogen, *Fusarium oxysporum* Schltd.:Fr. f. sp. *vasinfectum* (Atk.) W.C. Snyd. & H.N. Hans., have appeared in Australia (36), the United States (21,22), and the Ivory Coast (1). The Australian genotypes, thought to originate from native *F. oxysporum* associated with wild cottons (36), can cause heavy economic losses in cultivated cotton under wet and cool conditions (11). Despite years of intensive breeding efforts, only moderate host resistance is available against these genotypes (11). In California, Fusarium wilt caused by race 4 has become the dominant disease concern for cotton growers since its discovery in 2001 (12). In fact, a single resistant cultivar accounted for nearly three-quarters of Pima (*Gossypium barbadense* L.) cotton grown in that state in 2008 (35). Prior to its discovery in California, race 4 had been found only in Asia (3,16,30). In addition, four novel genotypes were recently reported from the southeastern United States, two of which ranked among the most virulent races in greenhouse trials (21). Lastly, multiple new genotypes of *F. oxysporum* f. sp. *vasinfectum* were found from a small sample of isolates collected from cotton in the Ivory Coast (1). Because this fungus is known to persist in acid delinted cottonseed (2,7,14,34), and is capable of surviving for decades in the soil as a saprophyte or in association with nonhosts (32,33), measures should be taken to prevent dissemination of infected seed from areas with virulent genotypes.

One potential approach to reducing seed infection by *Fusarium* is thermotherapy, which has been shown to significantly reduce seed infection by several other pathogenic fungi. Both dry heat and hot water treatments substantially reduced or eliminated *F. graminearum* (10,17), *Tilletia indica* (31), and *Neotyphodium* spp. (8) from wheat seed. *Fusarium moniliforme* was killed in maize caryopses by 15 min of immersion in water heated to 57 to 60°C (15). In another study, hot water treatments performed better than fungicides for controlling *Alternaria porri* and *Stemphylium vesicarium* in onion seed, although germination was reduced compared with nontreated seed (5). Nega et al. (27) developed hot water treatments effective against *Alternaria, Phoma,* and *Septoria* spp. infecting carrot, cabbage, celery, and parsley seeds. Similarly, Hermansen et al. (19) found *A. dauci* could be eliminated from carrot seed by submerging the seed for 20 min in water heated to 54°C. This hot water treatment did not adversely affect germination, seedling emergence, or yield (19). Hot water treatments have also been used to eliminate *Cladosporium variabile* and *Verticillium dahliae* from spinach seed (13). However, these treatments did not eliminate *Stemphylium botryosum* from highly infected seedlots (13).

Only a few studies have evaluated thermotherapy as a means of eliminating pathogens from cottonseed. Both hot water and dry heat treatments were examined for eliminating cotton anthracnose (*Glomerella gossypii*) from seed (6,25,26). Barre and Aull (6) reported that immersion of seed for 15 min in water at 70°C eliminated the anthracnose pathogen without reducing germination. Lehman (25) also found high-temperature dry heat treatments effective against *G. gossypii*.

Wide adoption of seed thermotherapy by the cotton industry will require not only that the treatments be effective, but also that those treatments do not reduce seed germination or vigor. Our objective was to test a range of low- and high-temperature dry heat treatments and short-duration hot water treatments for reducing or eliminating *F. oxysporum* f. sp. *vasinfectum* from cottonseed, and to evaluate the effects of the most promising treatments on seed germination and vigor.

MATERIALS AND METHODS

Dry heat treatments. Dry heat treatments were evaluated in two studies, one using relatively low temperatures (30 to 40°C), and one using higher temperatures (60 to 80°C). The experiment evaluating low temperatures used a naturally infected, acid delinted seedlot of the Upland (*G. hirsutum* L.) cultivar Phytogen 485 WRF, harvested from Louisiana in 2007. Harvested seed was stored at 4°C to maintain

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seed infection levels until experiments commenced. All dry heat treatments were conducted in incubators in which temperatures were monitored with HOBO recorders (Onset, Pocasset, MA). The four temperatures evaluated were 30, 35, and 40°C, and room temperature (25 to 27°C). In each of two repetitions (trials) of the experiment, the initial incidence of seed infection with *Fusarium* spp. was estimated by assaying 500 nontreated seeds. Incidence of *Fusarium* seed infection corresponding to each experimental temperature was evaluated weekly for 24 weeks. Groups of 50 seeds were put into 8 × 14 cm paper seed packets (Midco Enterprises, Inc., Kirkwood, MO); seed packet was the experimental unit. Three packets of seeds (replications) were randomly assigned to each temperature–time combination, for a total of 288 seed packets in each trial of the experiment.

Incidence of seed infection was determined by surface-sterilizing seeds with 95% ethanol and 0.625% sodium hypochlorite as described previously (7), and assaying the seeds on malachite green agar media (9). Five plates of malachite green agar were used per packet; therefore, plate was considered a subsample of packet. Plates were incubated under a 14-h photoperiod provided by fluorescent lights. Seeds were evaluated for presence of *Fusarium* spp. after 7 to 9 days of incubation. Most of the seed-derived fusaria had conidial morphology typical of *F. oxysporum,* but some appeared slightly different. All fusaria were counted for the analyses because of the difficulty in discriminating *F. oxysporum* from other fusaria. Seeds in packets were assayed within 4 days of removal from incubators.

The experiment evaluating high temperatures used seed artificially infected with race 4. To generate infected seed, cotton bolls were inoculated with a conidial suspension of the race 4 isolate FOV14 (22). The conidial suspension was produced by adding a conidial suspension of the isolate to flasks containing ¼-strength potato dextrose broth (BD Difco, Franklin Lakes, NJ), and continuously shaken at 125 rpm on an orbital shaker for 3 to 7 days. Spores were filtered through eight layers of cheesecloth, quantified using an Improved Neubauer hemacytometer (Hausser Scientific, Horsham, PA), and diluted to 1×10^4 spores/ml with sterile distilled water. A commercial field of the Pima cultivar Cobalt (Bayer CropScience, Research Triangle Park, NC) was used for inoculations in 2007. Hard green cotton bolls, at least 3.5 cm in diameter, were stabbed once between seams of the carpel wall with a dissecting needle (model 320-225, Walter Stern, Port Washington, NY) to a depth of 4 mm. Tubes with conidial suspensions were mixed by inversion, and 2 µl of the suspension (~20 spores) was inserted into the wound using a pipette. After bolls matured, seed cotton was hand harvested and ginned. Ginned seeds were delinted in 50 ml of concentrated sulfuric acid per 150 g of seed. Seeds were stirred in the acid until linters dissolved, which required approximately 1 min. Seeds were rinsed of acid with running tap water, neutralized in 0.75 M $NaHCO_3$, and rinsed again. Rinsed and neutralized seeds were dried in wire-mesh trays for approximately 25 min in a custom-made seed dryer. The seed dryer generated forced air heated to 43°C through a modified space heater. After seeds were dried, discolored and misshapen seeds were removed by hand, and remaining seeds were stored at 4°C.

Two trials of the high-temperature experiment were conducted within 6 months of seed harvest. The four temperatures evaluated were 60, 70, and 80°C, and room temperature (25 to 27°C). Prior to each repetition of the experiment, 300 seeds were assayed to determine initial incidence of seed infection. Incidence of *Fusarium* seed infection corresponding to each temperature was determined every 2 days for 14 days. As for the low-temperature experiment, seeds were placed into packets, and three packets were randomly assigned to each temperature–time combination. Therefore, a total of 84 packets were used in each trial of the experiment. Seeds were assayed for all fusaria within 4 days of removing packets from incubators as for the low-temperature dry heat experiment.

Hot water treatments. Hot water treatments were conducted on race 4–infected seeds of the Upland cultivar Daytona RF (Bayer CropScience) harvested in 2008. The seeds were artificially infected, delinted, and placed into seed packets as for the high-temperature dry heat treatments, except that four packets were randomly assigned to each treatment interval. Eleven treatment times were evaluated: 45 to 180 s, in intervals of 15 s, and a nontreated control. Thus, a total of 44 packets were used for each of the two trials of the experiment.

Distilled water was heated to 90°C (±1.5°C) in a 12-liter water bath. Water temperature was monitored with an alcohol thermometer. Seeds were removed from individual packets, placed in stainless steel strainers, and immersed in the hot water. After treatment, seeds were shaken onto clean plastic weigh boats lined with paper towels inside a laminar flow hood. Each group of 50 seeds was air-dried in weigh boats for 3 to 7 days. In preliminary tests, immersed seeds returned to their preimmersion weights after 3 days of air-drying (R. S. Bennett, *unpublished data*). To assay for *Fusarium* infection, 10 treated seeds were placed on each of five plates of Komada's medium (24). Seeds from control packets were surface-sterilized prior to plating, but immersed seeds were plated without further treatment. Plates were monitored every 2 to 3 days, and if *Fusarium*-infected seeds were present, uninfected seeds were transferred to a new plate of Komada's medium. Because seeds were occasionally infected with multiple fungi, including fusaria, seeds infected with other fungi were incubated until non-*Fusarium* colonies were approximately 2.5 cm in diameter, or a *Fusarium* co-infection was observed. At that point, seed status (infected with fusaria or not) was recorded and seeds were discarded. Seeds were monitored for a maximum of 14 days after plating.

Germination and vigor evaluations. Germination and vigor assays were conducted using acid delinted seed of cultivars Cobalt and Daytona not treated with fungicides or insecticides. The Cobalt and Daytona seed were from commercial seedlots harvested from fields free of Fusarium wilt in 2008. Germination and vigor assays were conducted following AOSA guidelines (4) for a subset of the high-temperature dry heat treatments and all of the hot water treatments. Because preliminary experiments indicated that high-temperature dry heat treatments longer than 8 days severely reduced seed germination, only seeds exposed to high temperatures for 2, 4, 6, and 8 days were evaluated. Four packets of seed were used for each temperature/time combination in both the germination and vigor assays. Therefore, a total of 64 and 44 packets were used to assay each cultivar in each trial of the dry heat and hot water treatments, respectively. All assays were conducted twice.

A 25.4 × 38 cm sheet of heavy weight germination paper (Anchor Paper Company, St. Paul, MN) was dipped in sterile distilled water and pressed onto a dry germination sheet to remove excess moisture. The moistened sheet was placed on a sheet of wax paper of the same size and evenly sprayed with 8 ml of a chlorothalonil suspension (4 ml of 29.6% a.i. formulation per liter of water) (Daconil Fungicide Concentrate, GardenTech, Lexington, KY) to suppress growth of fungi. Seeds from a single packet were spaced evenly on the sheet using a seed counting tray (Hoffman Manufacturing, Jefferson, OR). The seeds were sprayed with an additional 8 ml of the chlorothalonil suspension and covered with a second sheet of moistened germination paper. The sheets of paper containing seeds were folded along the longest dimension to produce a 2-in.-wide roll with the wax paper exposed. The germination rolls were secured with rubber bands and placed into polyethylene bags vented with 0.63-cm-diameter holes spaced approximately 14 cm apart. The polyethylene bags were placed in growth chambers (Conviron PGR15, Controlled Environments Ltd., Winnipeg, Canada) so that the rolls stood upright. Growth chambers were set to a 14-h photoperiod provided by fluorescent lights. Temperature was set at 30°C for the

standard germination assay and at 18°C for the vigor assay.

Seed germination was evaluated at 4 and 8 days for the standard germination test and at 7 days for the vigor test. The number of seedlings that grew to a length of 4 cm or longer from the primary root tip to the base of the cotyledon were counted and removed from the roll. If ungerminated seeds and short seedlings remained after the 4-day evaluation at 30°C, the papers were re-rolled and returned to the growth chamber for reevaluation at 8 days.

Experimental design and data analyses. Data from all experiments were analyzed by mixed-model ANOVA using PROC GLIMMIX of SAS (SAS, ver. 9.2, SAS Institute, Cary, NC), and untransformed data are presented. In the low-temperature dry heat experiment, the response variable was the number of seeds exhibiting fusaria. Fixed effects included temperature, duration of exposure in weeks, and their interaction. Trial (repetition of the experiment) and replication (packets) were used as random effects. Since five plates of seeds were assayed for each packet, plates were considered subsamples. Therefore, the random effect of trial*week*temperature*packet was included as the error term for testing the main effects and their interaction. The SLICE option of the LSMEANS statement was used to examine the interaction of temperature and week of exposure. Simple effects of temperatures within each week, and week within each temperature, were compared using the SLICEDIFF option of the LSMEANS statement. In each set of multiple comparisons, Type I error was controlled at $\alpha = 0.05$ using the ADJUST=SIMULATE option.

Initial analyses of the high-temperature dry heat experiment revealed problems with heterogeneity of variance that were not resolved through transformation. Therefore, instead of using plates as subsamples, means of the numbers of seeds with fusaria in each plate assigned to a given packet were analyzed. Fixed effects were temperature, duration of exposure in days, and their interaction. Trial was used as the random effect. Interactions between temperature and days of exposure were analyzed as for the low-temperature experiment.

For the hot water experiment, the response variable was the number of seeds exhibiting fusaria. The fixed effect was duration of immersion in seconds, and the random effects were trial and packet. Because the five plates per packet were used as subsamples, the random effect of trial*packet*immersion time was included as the error term for testing the effects of immersion time. Seed infection levels were compared among immersion times using the LSMEANS statement. Type I error was controlled as for the dry heat experiments.

For the seed germination and vigor experiments, the response variable was the proportion of seeds that germinated normally, as defined by AOSA guidelines (4). Heterogeneity of variance was observed in the data so the proportion of seeds germinated was arcsine-square root transformed. Separate analyses were conducted for each combination of cotton cultivar (Cobalt and Daytona) and germination temperature (30°C, standard germination; 18°C, vigor). In each analysis, random effects were trial and packet, and interactions between temperature and days of exposure were analyzed as for the dry heat and hot water experiments. Results of analyses on transformed proportions are presented as untransformed means.

RESULTS

Dry heat treatments. The naturally infected seedlot used for the low-temperature

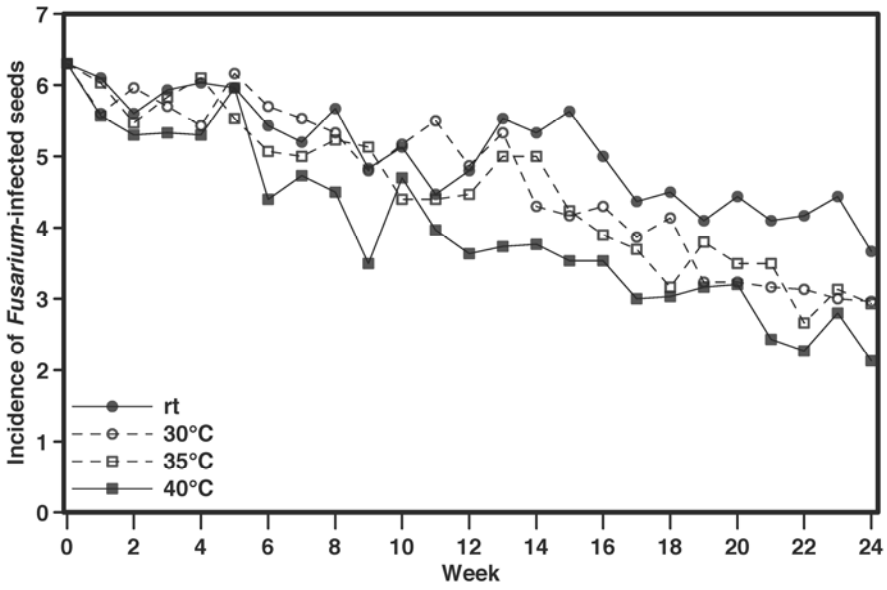

Fig. 1. Influence of seed incubation temperature (room temperature, 30, 35, 40°C) and exposure time (1 to 24 weeks) on incidence of *Fusarium*-infected seeds exposed to low-temperature dry heat. Initial infection (time 0) is the mean incidence of infection in 500 nontreated seeds at the beginning of heat treatment. For each subsequent treatment/time combination, 150 seeds were assayed for each of the two trials of the experiment.

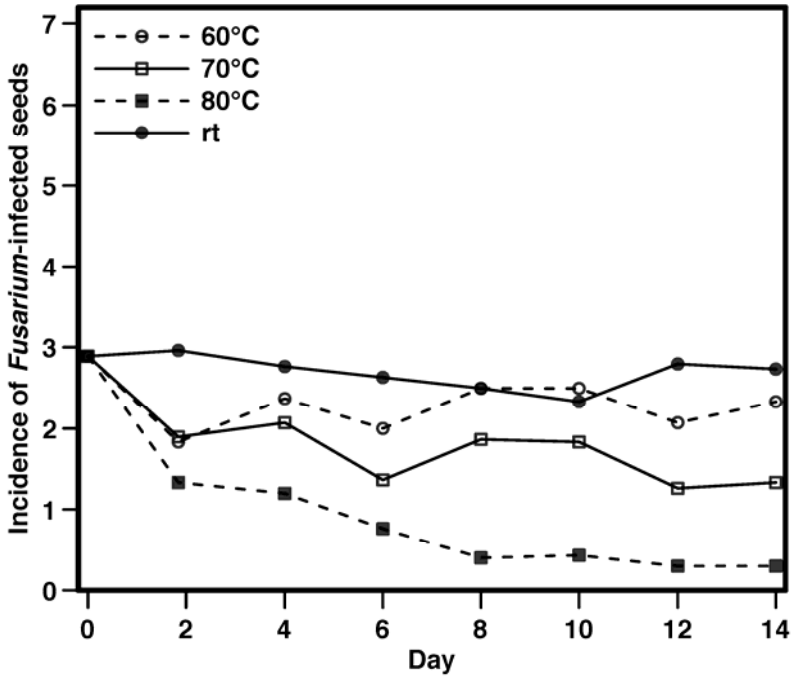

Fig. 2. Influence of seed incubation temperature (room temperature, 60, 70, 80°C) and exposure time (2 to 14 days) on incidence of *Fusarium*-infected seeds exposed to high-temperature dry heat. Initial infection (time 0) is the mean incidence of infection in 300 nontreated seeds at the beginning of each trial. For each subsequent treatment/time combination, 150 seeds were assayed for each of the two trials of the experiment.

dry heat experiment was highly infected with *Fusarium* spp. (Fig. 1). Although tests of both treatment temperature (F = 56.23; df = 3, 479; $P < 0.001$) and duration (F = 37.37; df = 23, 479; $P < 0.001$) were statistically significant, the significant interaction (F = 1.5; df = 69, 479; $P < 0.009$) indicated the respective effects of temperature and duration were dependent on the level of the other factor. Slices of the interaction indicated seed infection declined more rapidly at higher temperatures than at lower temperatures. For example, seed infection declined significantly between week 1 and weeks 11 and 15 for the 40°C (P = 0.042) and 35°C (P = 0.007) treatments, respectively. However, a significant decline in seed infection from week 1 levels was not observed for the room temperature and 30°C treatments until week 17 (room temperature, P = 0.014; 30°C, P = 0.012). Although the number of seeds infected with *Fusarium* declined over time, infection was not eliminated by 24 weeks of exposure to any of the temperature treatments (Fig. 1). When the heat treatments were compared to the room temperature control using the simple effects of temperature treatments over time, only the 40°C treatment had fewer fusaria than the control at most durations of exposure. Seed infection levels in the 40°C treatment first differed from those in the control at week 8 (P = 0.0346); thereafter, infection levels in the 40°C treatment differed from those at room temperature except at weeks 10, 11, and 19. Seed infection levels in the 30 and 35°C treatments differed significantly from those at room temperature only at weeks 20 and 23, and weeks 15 and 18, respectively.

In the high-temperature dry heat treatment, approximately 30% of the seed harvested from inoculated bolls were infected with fusaria (Fig. 2). Preliminary tests of the inoculation method in which fusaria were screened with race 4–specific primers (7) indicated the majority of fusaria from seeds of inoculated bolls were race 4 (R. S. Bennett, *unpublished data*). Despite being infected with fusaria, 38% of the infected seeds germinated on the assay plates over both trials of the high-temperature dry heat experiment. The interaction between treatment temperature and duration of exposure was significant (F = 2.33; df = 18, 139; P = 0.003) in the high-temperature experiment, and slices of the interaction indicated that higher temperatures reduced seed infection more rapidly than lower temperatures. Seeds incubated at 80°C had significantly less seed infection than seeds held at room temperature ($P < 0.001$, Fig. 2) on all treatment days. However, fusaria were not eliminated from seed even after 14 days of incubation at 80°C. Seeds incubated at 70°C had significantly less seed infection than seeds held at room temperature on days 2, 6, 12, and 14, but had significantly more seed infection than seeds incubated at 80°C on days 4, 8, 10, 12, and 14. Seed infection levels in the 60°C treatment differed from the room temperature treatment only on day 2.

Hot water treatments. In the hot water experiment, approximately 56% of the seed harvested from bolls inoculated with spores of race 4 were infected with fusaria (Fig. 3). The number of *Fusarium*-infected seeds declined with duration of immersion in hot water (F = 33.89; df = 10, 76; $P < 0.001$). All hot water treatments differed significantly in seed infection from the nontreated control ($P < 0.001$). Nontreated seed ($P < 0.001$) and seed immersed for 45 s (P = 0.001) and 60 s (P = 0.043) exhibited significantly higher levels of seed infection than seed immersed for 180 s. However, the incidence of *Fusarium*-infected seed did not differ significantly among immersion times ≥75 s.

Germination assays. The interaction between temperature and duration of high-temperature dry heat treatment was significant for both germination and vigor (Table 1), indicating that the respective effects of temperature and duration were dependent on the level of the other factor. In the high-temperature dry heat treatments, seed germination (Fig. 4A and C) and vigor (Fig. 4B and D) were not significantly reduced relative to the nontreated seed, except for all durations of the 80°C treatment, and for 6 and 8 days of exposure at 70°C. For the 80°C treatment, a significant reduction in both germination and vigor in Cobalt seed (Fig. 4A and B, respectively) was observed after 4 days of incubation compared with seeds held at room temperature (germination, P = 0.025; vigor, $P < 0.001$). In the 70°C treatment, Cobalt seed lost germination (Fig. 4A; P = 0.028) and seed vigor (Fig. 4B; $P < 0.001$) by day 6 relative to the nontreated seed. Germination of Daytona seed treated at 80°C declined by day 2 (Fig. 4C; P = 0.06), but the decline was not statistically significant until day 4 (Fig. 4C; P = 0.007). Vigor of the Daytona seed treated at 80°C was reduced by day 2 (Fig. 4D; $P < 0.001$). At 70°C, germination of Daytona seed did not decline by day 8 (Fig. 4C; P = 0.781) relative to the nontreated seed, and seed vigor was reduced only at day 4 (Fig. 4D; P = 0.023).

The duration of immersion in hot water also had a significant effect on both germination and vigor (Table 1). Nonetheless, seed of both cultivars could be immersed in 90°C water for up to 105 s without significant loss of germination or vigor compared with nontreated seed (Fig. 5). Seed of Cobalt exhibited a statistically significant reduction in germination and vigor after 120 s of immersion (germination, P = 0.016; vigor, $P < 0.001$) compared to the nontreated control. Daytona seed did not demonstrate a reduction in germination or vigor compared to the nontreated seeds until seeds were immersed for ≥150 s (germination, P = 0.039; vigor, $P < 0.001$).

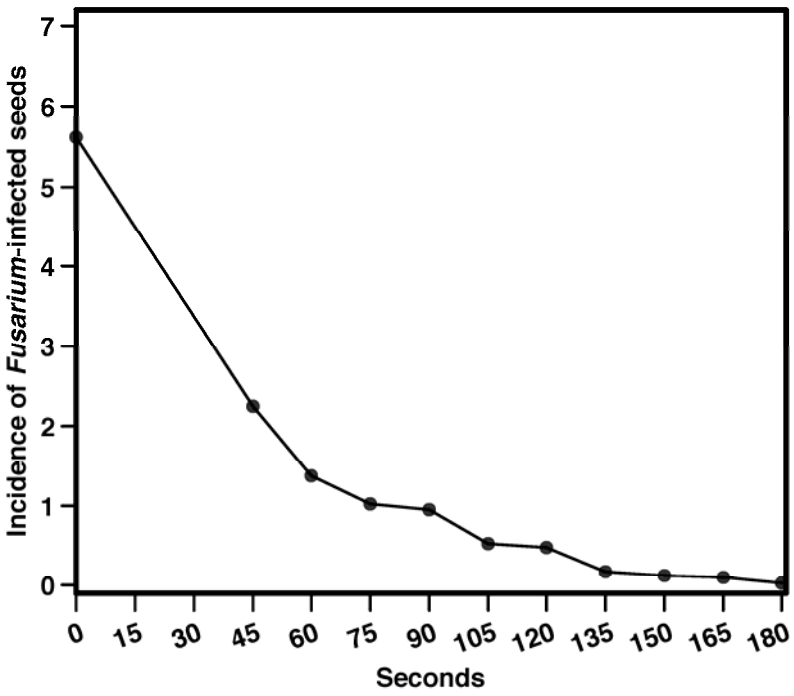

Fig. 3. Influence of immersion time (45 s to 3 min) in 90°C water on incidence of *Fusarium*-infected seeds. Incidence of infection at time 0 is the mean incidence in seed not immersed in hot water. Two-hundred seeds were assayed in each of two trials of the experiment for each time period.

DISCUSSION

Of the thermotherapy treatments tested here, 90°C hot water appeared most effective at removing fusaria from cottonseed with minimal loss of germination and seed vigor. The incidence of *Fusarium*-infected

seeds dropped from above 50% to less than 10% in seed immersed for 105 s. This reduction in seed infection was achieved without a statistically significant loss of seed germination or vigor. A significant reduction in seed infection was also observed in seed exposed to 80°C dry heat for 6 days, but this treatment resulted in seed injury. Seed germination and vigor of the cultivar Cobalt was reduced after only 4 days of incubation at 80°C. Seed vigor was reduced in the cultivar Daytona after only 2 days at 80°C. The 70 and 60°C dry heat treatments were not as effective as the 80°C treatment, and none of the low-temperature dry heat treatments was able to reduce seed infection below 20% after 24 weeks. Seed stored at room temperature also showed a decline in seed infection, albeit slowly, as previously reported (14,23).

There are potential obstacles to commercial adoption of these thermotherapy treatments. While the hot water treatments appeared promising, seed coat separation from the chalaza was observed after all immersion times. The seed coat appeared normal after the 3- to 7-day drying period, but peeling seed coats might prevent integration of thermotherapy into existing acid delinting operations. Fungicides and insecticides are usually applied to seed immediately after delinting. It may be possible to prevent seed coat alteration or damage by using alternative heating liquids such as vegetable or petroleum-based oils (29,37). Zinnen and Sinclair found that the seed-coat of soybean seeds sloughed off in water but remained intact when heat-treated in vegetable oil (37). Regardless of the liquid medium chosen for heat treatment, an additional step to dry the seed would be necessary. For the high-temperature dry heat treatments, the duration of exposure required for significant reduction in seed infection is likely impractical for integration into commercial operations. High-temperature dry heat treatments may be

Table 1. Effects of high-temperature dry heat (2, 4, 6, and 8 days exposure) and 90°C hot water treatments (45 to 180 s) on germination of cotton seed from cultivars Cobalt and Daytona

Experiment	Cultivar	Assay[a]	Model effect[b]	df	*F*	*P*
Dry heat	Cobalt	Germination	Temperature	3, 105	158.02	<0.001
			Day	3, 105	73.32	<0.001
			Temperature × time	9, 105	36.37	<0.001
	Cobalt	Vigor	Temperature	3, 111	257.42	<0.001
			Day	3, 111	76.11	<0.001
			Temperature × time	9, 111	36.27	<0.001
	Daytona	Germination	Temperature	3, 111	33.35	<0.001
			Day	3, 111	5.86	<0.001
			Temperature × time	9, 111	2.28	0.022
	Daytona	Vigor	Temperature	3, 112	161.73	<0.001
			Day	3, 112	11.35	<0.001
			Temperature × time	9, 112	9.19	<0.001
Hot water	Cobalt	Germination	Seconds	10, 70	16.57	<0.001
	Cobalt	Vigor	Seconds	10, 70	24.66	<0.001
	Daytona	Germination	Seconds	10, 70	17.14	<0.001
	Daytona	Vigor	Seconds	10, 70	7.31	<0.001

[a] Temperatures were 30°C for the standard germination assay and 18°C for the seed vigor assay.
[b] Mixed-model ANOVA was used to analyze arcsine-square root transformed proportion of seeds germinated.

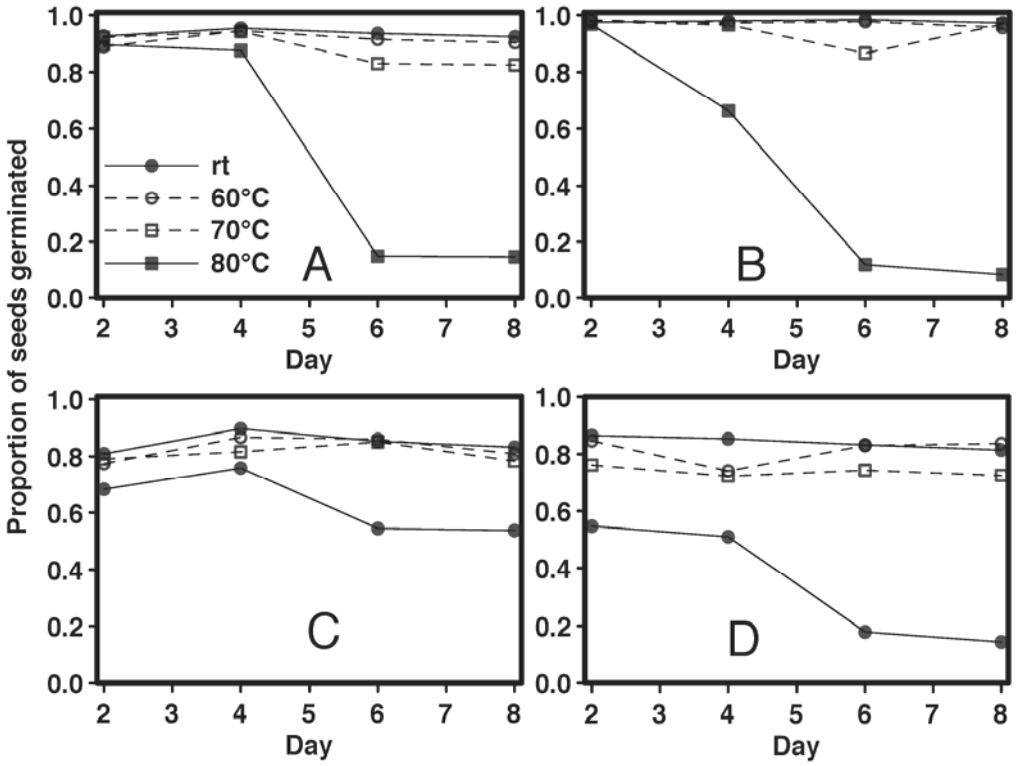

Fig. 4. Influence of seed incubation temperature (room temperature, 60, 70, 80°C) and exposure time (2 to 8 days) on seed germination **(A, C)** and vigor **(B, D)** of Pima cultivar Cobalt **(A, B)** and Upland cultivar Daytona **(C, D)**.

useful to smaller-scale applications, such as the exchange of potentially infected seed for breeding purposes. However, more work is needed to determine if the loss in seed germination and vigor from the high dry heat regimens can be avoided by controlling for factors such as seed moisture and age (18,37). The Cobalt and Daytona seed used here were from the most recent harvest, but seed moisture was not measured. Lastly, the results suggest Cobalt and Daytona cultivars may differ in their ability to tolerate heat treatment. Multiple seedlots of additional Pima and Upland cultivars will need to be directly compared to determine if heat treatments should be customized for each cultivar.

Considerable levels of infection (up to 47%) by *F. oxysporum* f. sp. *vasinfectum* have been reported in naturally infected seedlots of cotton (2,20,28). Nonetheless, one significant obstacle to testing seed treatments has been the difficulty of obtaining seedlots that are highly infected with *F. oxysporum* f. sp. *vasinfectum*, particularly from dry seasons or production areas. We were able to circumvent this problem by inoculating bolls. While substantial proportions of infected seed from inoculated bolls were dead, a significant fraction of infected seed germinated, possibly indicating that infected seeds derived from inoculated bolls are reasonable substitutes for naturally infected seed. Further studies will use this technique to optimize hot water treatments with the goal of obtaining maximum reduction of *F. oxysporum* f. sp. *vasinfectum* with minimum seed damage for a broad selection of cultivars.

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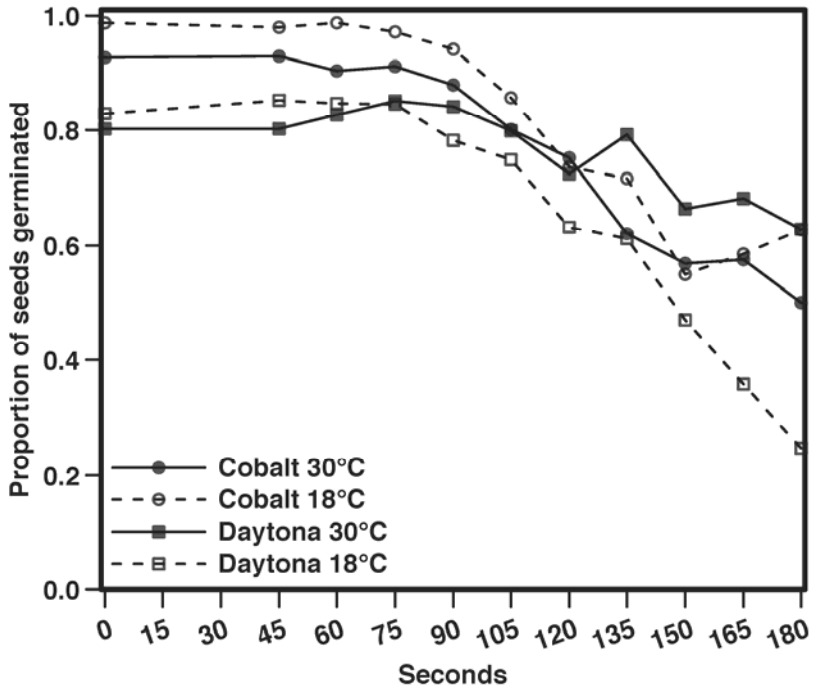

Fig. 5. Influence of immersion time (45 s to 3 min) in 90°C water on seed germination (30°C) and vigor (18°C) of Pima cultivar Cobalt and Upland cultivar Daytona.

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